

VISIT...

LANZAROTE
Caliente.COM

Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies

Darlene C. Deecher¹, Milton Teitler^{1,*}, David M. Soderlund², William G. Bornmann³,
Martin E. Kuchne³ and Stanley D. Glick¹

¹Department of Pharmacology and Toxicology, Albany Medical College and Capital District Center for Drug Abuse Research and Treatment, Albany, NY 12208 (U.S.A.), ²Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456 (U.S.A.) and ³Department of Chemistry, University of Vermont, Burlington, VT 05405 (U.S.A.)

(Accepted 17 September 1991)

Key words: Ibogaine; Harmaline; κ -Receptor; Opiate receptor; Sodium channel; γ -Aminobutyric acid receptor

Assays using radioligands were used to assess the actions of ibogaine and harmaline on various receptor types. Ibogaine congeners showed affinity for opiate receptors whereas harmaline and harmine did not. The K_i for coronaridine was 2.0 μ M at μ -opiate receptors. The K_s for coronaridine and tabernanthine at the δ -opiate receptors were 8.1 and 3.1 μ M, respectively. Ibogaine, ibogamine, coronaridine and tabernanthine had K_i values of 2.08, 2.6, 4.3 and 0.15 μ M, respectively, for κ -opiate receptors. Long-lasting, dose-dependent behavioral effects of ibogaine have been reported. The possibility that these effects were due to irreversible binding properties of ibogaine at κ -receptors was considered; however, radioligand wash experiments showed a rapid recovery of radioligand binding after one wash. A voltage-dependent sodium channel radioligand demonstrated K_i values in the μ M range for all drugs tested. Using radioligand binding assays and/or ³⁶Cl⁻ uptake studies, no interaction of ibogaine or harmaline with the GABA receptor-ionophore was found. The κ -activity of ibogaine (or an active metabolite) may be responsible for its putative anti-addictive properties whereas the tremorigenic properties of ibogaine and harmaline may be due to their effects on sodium channels.

INTRODUCTION

Ibogaine and harmaline, indole alkaloids, are part of a large group of centrally acting drugs that produce tremorigenic and hallucinogenic effects^{2,24,40}; ibogaine is also under investigation for potential use in the treatment of opiate addiction (U.S. Patent 4,499,096). The mechanism of action of either of these compounds is not clearly understood. Affinities of harmaline analogs at opiate receptors have been reported²³; it is possible that ibogaine analogs might also have affinity for these receptors and appropriate studies were therefore conducted.

Published reports have indicated that the tremorigenic properties of ibogaine and harmaline analogs may be mediated through the GABA receptor-ionophore complex^{15,28,29,36,38}; our studies were designed to investigate this hypothesis further. It is also conceivable that tremors are induced by interference with ion conductance or membrane potentials, since other tremorigenic agents demonstrate affinity for voltage-dependent sodium chan-

nels²⁷; the interactions of ibogaine and harmaline were investigated at this site.

Many drugs demonstrating hallucinogenic properties have been shown to be mediated through agonist interactions at the 5-HT₂ receptor¹. Since ibogaine²⁴ and harmaline²⁵ have been reported to produce hallucinations, 5-HT₂ interactions were examined.

Using in vitro radioligand binding and functional ³⁶Cl⁻ uptake assays, we investigated mechanisms of action that may be responsible for the hallucinogenic, tremorigenic and proposed anti-addictive effects of ibogaine and harmaline analogs.

MATERIALS AND METHODS

Membrane preparation

Neuronal homogenates used in all assays except for studies of ³⁶Cl⁻ uptake and voltage-dependent sodium channels were prepared as followed. Bovine or rat tissue was obtained by dissection and immediately homogenized in 50 mM Tris-HCl buffer (pH 7.7 at 25°C) containing 10 mM MgSO₄ and 0.5 mM EDTA. Following centrifugation for 15 min at 14,000 g the samples were resuspended in buffer and preincubated for 15 min at 37°C to destroy any en-

* Formerly spelled Teitler.

Correspondence: D.C. Deecher, Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208, U.S.A. Fax: (1) (518) 445-5799.

dogenous ligands. The homogenates were centrifuged for 10 min at 14,000 g, resuspended in buffer and recentrifuged before storage at -30°C . Mouse brain vesicles were freshly prepared in 20 mM HEPES for $^{36}\text{Cl}^{-}$ uptake studies following standard procedures⁵. Mouse brain vesicles prepared for radiolabeled [^3H]BTX-B assays were made in 50 mM HEPES following published procedures⁹. Protein determinations were performed using the Pierce BCA protein assay³².

Chemicals

Ibogaine hydrochloride (HCl), harmaline HCl, harmaline HCl, harmine HCl and naloxone HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Racemic coronaridine and racemic ibogamine were synthesized by Martin Kuehne and William Bornmann, University of Vermont, Burlington, VT. Tabernanthine was supplied by P. Potier, C.N.R.S., Institute of Chemistry of Natural Substances, Gif-sur-Yvette, France. Except for [^3H]Carfentanil (80 Ci/mmol), which was custom synthesized by NEN DuPont, Boston, MA, the [^3H]radioligands used are commercially available from NEN DuPont, Wilmington, DE. In $^{36}\text{Cl}^{-}$ uptake studies ibogaine and harmaline were prepared and delivered in 0.4 μl DMSO.

Radioligand binding assays

Assays were performed in triplicate for competition studies in 2.0 ml volume containing 800–900 μl buffer, 100 μl non-specific determinant, 100 μl radioligand and 1 ml tissue homogenate. Positive controls using drugs with known affinities for each receptor site were run in parallel in each drug screening. Tubes were incubated under specific conditions (Table I) and filtered through Whatman GF/B glass fiber filters with 10 ml cold 50 mM Tris-HCl buffer. The filters were counted by liquid scintillation spectrometry. Assay conditions for [^3H]BTX-B were done as previously described⁹. Specific binding was defined as binding in the presence of a saturating concentration of competing ligand. Ibogaine or harmaline analogs were considered inactive at the defined receptor site if there was lack of inhibition at concentrations above 100 μM .

$^{36}\text{Cl}^{-}$ uptake assays

Assay conditions for $^{36}\text{Cl}^{-}$ uptake studies were done following standard procedures⁵. Specific GABA-stimulated uptake was calculated as the difference between basal uptake and total uptake in the presence of GABA (final concentration 20 or 100 μM). Inhibition of stimulated uptake was determined by comparing $^{36}\text{Cl}^{-}$ uptake in the presence of GABA (100 μM) and either ibogaine or harmaline (100 μM) to that obtained with GABA alone. A known inhibitor of $^{36}\text{Cl}^{-}$ uptake, endrin (10 μM), was used as a positive control.

Wash experiment

Centrifuge tubes containing 30 ml of bovine homogenates (282 $\pm$ 31 $\mu\text{g}/\text{ml}$ protein) were incubated with either 50% (v/v) ethanol, 10 nM U-69593 ($K_i \times 10$) or 20 μM ibogaine ($K_i \times 10$) for 30 min at 37°C . After incubation each tube was vortexed, a 20- μl sample taken for protein determination, and triplicate 1-ml aliquots were taken for each set of total and non-specific samples. The volume was restored with buffer to the original tubes, centrifuged at 14,000 g for 10 min, supernatant removed and the membrane resuspended in 26 ml buffer. One-ml aliquots were taken from the centrifuge tubes and the volume restored to 30 ml. This wash procedure was repeated 3 times. After the last wash procedure the last group of 1-ml aliquots were taken and the binding assay performed. Total volume of each test tube contained 800 or 900 μl 50 mM Tris buffer, 100 μl [^3H]U-69593, 1 ml bovine neuronal membranes and 100 μl (1 μM) naloxone (non-specific determinant). The reaction was initiated by addition of [^3H]U-69593 and the test tubes incubated for 30 min at 37°C . The reaction was terminated by rapid vacuum filtration using Whatman GF/B glass fiber filters, and washed with 10 ml cold 50 mM Tris buffer. The filters were counted by liquid scintillation spectrometry in 5 ml ecoscint (National Diagnostics). Protein determination was performed using the Pierce BCA assay.

RESULTS

Our initial studies evaluated the potencies of ibogaine and harmaline for a series of radioligands known to label a variety of neuroreceptors. Except for the interaction of ibogaine at the κ -opiate receptor, neither ibogaine nor harmaline had significant affinities ($K_i > 100 \mu\text{M}$) in any of the assays listed (Table I). The lack of affinity of ibogaine or harmaline for serotonin (5-HT) receptors suggests that the hallucinogenic properties are not being mediated by the 5-HT₂ receptor subtype. Ibogaine's affinity for the κ -opiate receptor was in the μM range (Fig. 1). Affinities for opiate receptors were demonstrated by ibogaine analogs, but none were shown by harmaline analogs (Table II). Because of previous reports regarding ibogaine's long-lasting drug effects, radioligand wash

TABLE I

Survey of receptor affinity for ibogaine and harmaline using radiolabeled binding assays.

Assay conditions followed references cited; all data are representative of triplicate determinations from 3 experiments using different membrane preparations.

Receptor	[^3H]Ligand	Tissue	Refs.
Adrenergic			
α_1	prazosin	rat cortex bovine cortex	13
α_2	p-amino-clonidine	rat cortex	30
β_1	dihydro-alprenolol HCl	calf cortex	18
Cannabinoid	CP55,940 Win5212-2	calf cerebellum calf cerebellum	11
Dopamine			
D ₁	SCH23390	rat striatum calf caudate	14 31
D ₂	N-methyl-spiperone	rat striatum calf striatum	19
GABA			
A	muscimol	bovine cortex bovine cerebellum	3
BZD	flunitrazepam	rat cortex bovine cortex	6
Cl ⁻ channel	$^{36}\text{Cl}^{-}$	mouse synaptoneurosome	5
Muscarinic	QNB	bovine cortex bovine hippocampus	37
Nicotinic	methylcarbaryl-choline	bovine cortex	1
Opiate			
μ	carfentanil	bovine cortex	35
δ	DPDPE	bovine cortex	7
κ	U69,593	bovine cortex	17
Serotonin			
1 _a	DPAT	Rat hippocampus	33
1 _b	5-HT	rat striatum	33
1 _c	mesulergine	rat cortex	33
1 _d	5-HT	bovine caudate	8
2	ketanserin	rat cortex	34
3	GR65630	area postrema	22

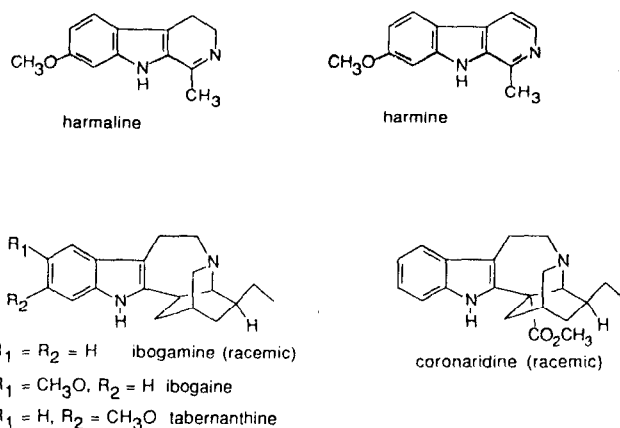


Fig. 1. Structures of harmaline and ibogaine analogs.

experiments were done to determine if irreversible properties of ibogaine at κ -opiate receptors could be demonstrated. Readily reversible binding of ibogaine was shown when competed with [3H]U69593, a specific κ -opiate agonist (Fig. 2).

It has been proposed that the tremorigenic effects of ibogaine and harmaline are mediated through the GABA receptor-ionophore complex. However, in competition studies, neither ibogaine nor harmaline showed inhibition of binding for [3H]muscimol or [3H]flunitrazepam, GABA_A and benzodiazepine specific radioligands, respectively. Functional studies of $^{36}Cl^-$ uptake were done next. Drug interactions at the chloride channel, benzodiazepine or GABA site associated with the GABA receptor-ionophore complex can be functionally determined by studying drug induced interactions in $^{36}Cl^-$ uptake. In our studies, neither ibogaine nor harmaline demonstrated any inhibitory effect on $^{36}Cl^-$ uptake (Fig. 3). Additional studies showed that neither ibogaine nor harmaline enhanced or stimulated $^{36}Cl^-$ uptake (data not shown). At the concentrations used in our studies, these results rule out any interactions of ibogaine or harmaline at the GABA-ionophore complex.

TABLE II

Affinities of ibogaine analogs for opiate receptors

Data are means ($\pm$ S.D.) of triplicate determinations from 3 experiments using different membrane preparations. EBDA analysis determined K_i values.

Compound	K_i 's (μM) $\pm$ S.D.		
	μ	δ	κ
Ibogaine	>100	>100	2.08 ± 0.23
Coronaridine	2.02 ± 0.29	8.1 ± 1.9	4.3 ± 0.33
Ibogamine	>100	>100	2.6 ± 0.45
Tabernanthine	>100	3.1 ± 0.05	0.15 ± 0.05

TABLE III

Affinities of ibogaine and harmaline analogs for voltage-dependent sodium channels.

Data are means ($\pm$ S.D.) of triplicate determinations from 3 experiments using different membrane preparations. EBDA analysis determined K_i values.

Compound	K_i 's (μM) $\pm$ S.D.
Ibogaine	8.1 ± 1.3
Ibogamine	7.7 ± 0.29
Tabernanthine	7.9 ± 0.39
Coronaridine	15.9 ± 2.0
Harmaline	13.9 ± 0.57
Harmine	11.5 ± 0.4

Since neither ibogaine nor harmaline showed affinity for the GABA receptor, we speculated that the tremorigenic effects could be evoked by interference with membrane potentials or ion conductance. Therefore, studies were done to determine whether ibogaine or harmaline interacted at voltage-dependent sodium channels using [3H]batrachotoxinin A 20- α -benzoate (BTX-B), a selective radioligand. All analogs tested in this study showed affinity for the voltage-dependent sodium channel in the μM range (Table III).

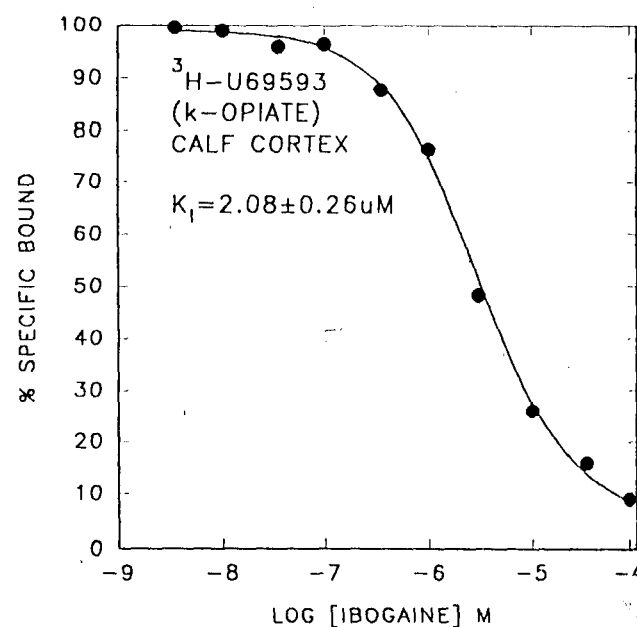


Fig. 2. Competition of ibogaine with [3H]U-69593 (1 nM) for the κ -opiate receptor. Data points represent triplicate determinations of 3 such experiments using different membrane preparations.

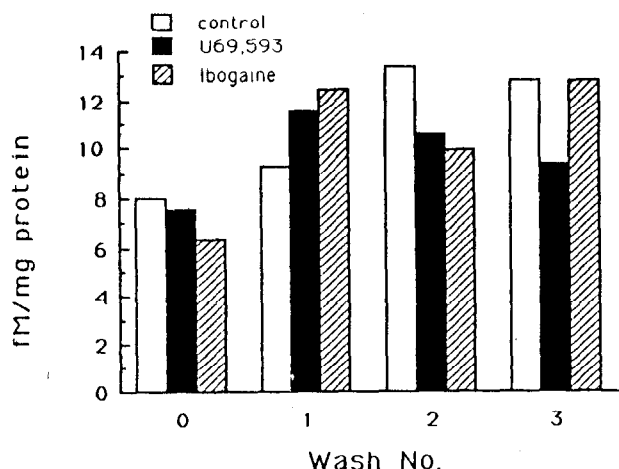


Fig. 3. Reversible binding characteristics of ibogaine at the κ -opiate receptor. Data are means of triplicate determinations from one experiment that is representative of 3 such experiments using different membrane preparations.

DISCUSSION

Results of our radiolabeled receptor survey demonstrate that ibogaine interacts at the κ -opiate receptor whereas harmaline does not. This κ -opiate interaction may explain the putative anti-addictive properties noted for ibogaine. Ibogaine has been shown to reduce morphine intake in rat self-administration experiments¹². The mechanism responsible for this effect is not known, but it is possible that it is mediated through a μ/κ -opiate receptor interaction. Buprenorphine has been shown to suppress cocaine and opiate drug dependency^{16,21}. The mechanism of action of buprenorphine is not clear but an interaction between μ - and κ -opiate receptors may be

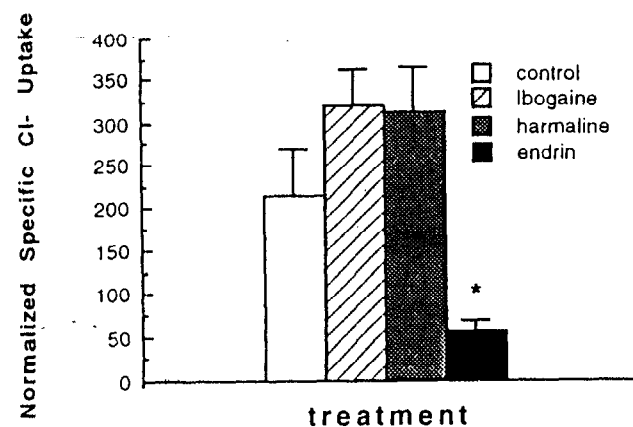


Fig. 4. The effect of preincubation with ibogaine (100 μ M), harmaline (100 μ M) and endrin (10 μ M) on GABA-stimulated ³⁶Cl uptake by mouse brain vesicles. Data are normalized for background ³⁶Cl⁻ uptake in the absence of drug. Bars show standard errors for 3 determinations and * was significant at $P < 0.05$ (paired t -test).

a contributing factor in its anti-addictive properties. Pre-treatment with naloxone can block the effects produced by buprenorphine but, if naloxone is administered after treatment with buprenorphine, then the effects cannot be blocked⁴. This suggests that the dissociation rate of buprenorphine is quite slow. Membrane wash experiments were carried out to determine whether ibogaine irreversibly or slowly dissociates from κ -opiate receptors. Although these membrane wash experiments do not provide precise kinetic measurements, they can be used to determine crude kinetic indices. Wash experiments showed a rapid recovery of competitive [³H]U-69593 binding after one series of membrane washing. Therefore, long-lasting behavioral effects of ibogaine^{12,20} are not due to an irreversible association at the κ -opiate receptor. However, we cannot rule out the possibility of an active metabolite whose half-life outlasts that of ibogaine, and which may also interact with κ -opiate receptors.

Published reports have indicated harmaline may act at the GABA receptor-ionophore complex^{15,28,29,38}. Results in our laboratory do not support this hypothesis. Neither ibogaine nor harmaline inhibited the binding of [³H]muscimol, a GABA_A agonist, thereby ruling out an effect at the GABA recognition site. The neuronal benzodiazepine (BZD) receptor site is also intimately associated with GABA receptors. Harmaline and ibogaine analogs both produce tremors indicating possible BZD receptor interaction⁴⁰. The β -carboline β -CCM, β -CCE and DMCM (methyl- β -carboline-3-carboxylate, ethyl- β -carboline-3-carboxylate and 6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylic acid methyl ester, respectively) are inverse BZD agonists²⁶. Since harmaline (1-methyl-7-methoxy-3,4-dihydro- β -carboline) is a β -carboline, it might be expected that harmaline would also demonstrate agonist properties at the BZD receptor. It has been reported that an analog of harmaline, harmene (ethyl-1-methyl- β -carboline-3-carboxylate) readily competed with [³H]flunitrazepam (FLU)²³, a specific agonist for the BZD binding site. Preliminary screening in our laboratory also showed harmene readily competed for (FLU) binding (data not shown). Therefore, [³H]FLU was used here as a radioligand to determine whether harmaline interacts with the BZD site. Our studies showed that neither ibogaine nor harmaline had any effect at this binding site. There are obvious structural differences between the β -carbolines that are known to act at the BZD site and harmaline. It has been speculated that the carboxylate group at the 3-position of the β -carboline structure is responsible for the interaction of β -CCM, β -CCE and DMCM at the BZD site³⁹. Harmaline does not possess the 3-carboxylate group, hence the lack of interaction at the BZD site. Another possible site

which ibogaine or harmaline could be acting through is the chloride ionophore within the GABA receptor-ionophore complex, which can be assessed by either [^{35}S]TBPS (*t*-butylbicyclo-phosphorothionate) binding or $^{36}\text{Cl}^-$ uptake studies. If ibogaine and harmaline act at this site, they should inhibit $^{36}\text{Cl}^-$ uptake, since agents acting at this Cl^- channel (TBPS, picrotoxinin and endrin) all act as channel blockers; however, neither ibogaine nor harmaline had any effect on inhibition or enhancement of Cl^- uptake suggesting a lack of interaction at the GABA receptor complex. Therefore, the tremorigenic properties of ibogaine and harmaline are not directly mediated via the GABA site.

We speculated that ibogaine- and harmaline-induced tremors may be the result of interference with ion conductance or membrane potentials. Classes of compounds such as the chlorinated hydrocarbons and dihydropyrazoles which demonstrate tremorigenic properties are known to elicit their effects through voltage-dependent sodium channels^{10,27}. In preliminary screening experiments ibogaine and harmaline analogs inhibited the binding of a voltage-dependent sodium channel-specific radioligand, [^3H]BTX-B, in mouse depolarized neuronal preparations. The inhibition of BTX-B binding indicates

an interaction of these compounds with voltage-dependent sodium channels. Further studies, such as functional sodium uptake and radioligand binding assays, are needed to determine whether these compounds are acting as sodium channel activators or blockers.

In summary, the results of our study indicate that ibogaine analogs show affinity for the κ -opiate receptor. The action of ibogaine, or an active metabolite, at this receptor may be responsible for its putative anti-addictive properties. Although tremorigenic effects of ibogaine and harmaline were thought to be mediated through the GABA receptor-ionophore complex, our radioligand binding and functional $^{36}\text{Cl}^-$ uptake studies did not support this hypothesis. It is possible that the tremorigenic effects are due to a disruption of ion conductance or membrane potential, since both ibogaine and harmaline effectively inhibited binding of a sodium channel-specific radioligand activator.

Acknowledgements. We would like to thank Dr. Gregory Payne (Cornell University, Geneva, NY) for assistance with the $^{36}\text{Cl}^-$ uptake assays. D.C.D. was supported by the Capital District Center for Drug Abuse Research and Treatment, Albany, NY, funded by the Aaron Diamond Foundation. The work was supported by NIDA Grant DA03817 to S.D.G. and MH-40716 to M.T.

REFERENCES

- 1 Abood, L.G., Grassi, S. and Noggle, H.D., Comparison of the binding of optically pure (-) and (+)-nicotine to rat brain membranes, *Neurochem. Res.*, 10 (1985) 259-267.
- 2 Airaksinen, M.M. and Kari, I., β -Carbolines, psychoactive compounds in the mammalian body, Part II: Effects, *Med. Biol.*, 59 (1981) 190-211.
- 3 Beaumont, K., Chilton, W.S., Yamamura, H.I. and Enna, S.J., Muscimul binding in rat brain: association with synaptic GABA receptors, *Brain Research*, 148 (1977) 153-162.
- 4 Bickel, W.K., Stitzer, M.L., Bigelow, G.E., Liebson, I.A., Jansinski, D.R. and Johnson, R.E., Buprenorphine: dose-related blockade of opioid challenge effects in opioid dependent subjects, *J. Pharmacol. Exp. Ther.*, 247 (1988) 47-53.
- 5 Bloomquist, J.R. and Soderlund, D.M., Neurotoxic insecticides inhibit GABA-dependent chloride uptake by mouse brain vesicles, *Biochem. Biophys. Res. Commun.*, 133 (1985) 37-43.
- 6 Braestrup, C. and Squires, R.F., Brain specific benzodiazepine receptor, *Br. J. Psychiatry*, 133 (1978) 249-260.
- 7 Clark, J.A., Itzhak, Y., Hraby, J., Yamamura, H.I. and Pasternak, G.W., [D-Pen², D-Pen⁵]enkephalin (DPDPE): a selective enkephalin with low affinity for μ -opiate binding sites, *Eur. J. Pharmacol.*, 128 (1986) 303-304.
- 8 Davis, K.H. and Titeler, M., Detection and characterization of the Serotonin 5HT_{1D} receptor in rat and human brain, *J. Neurochem.*, 50 (1988) 1624-1631.
- 9 Deecher, D.C., Payne, G.T. and Soderlund, D.M., Noncompetitive inhibition of [^3H]batrachotoxinin A-20- α -benzoate binding to mouse brain sodium channels by the dihydropyrazole insecticide RH3421, *Pestic. Biochem. Physiol.*, in press.
- 10 Deecher, D.C. and Soderlund, D.M., RH 3421, an insecticidal dihydropyrazole, inhibits sodium channel-dependent sodium uptake in mouse brain preparations, *Pestic. Biochem. Physiol.*, 39 (1991) 130-137.
- 11 Devane, W.A., Dysarz, F.A., Johnson, M.R., Melvin, L.S. and Howlett, A.C., Determination and characterization of a cannabinoid receptor in rat brain, *Mol. Pharmacol.*, 34 (1988) 605-615.
- 12 Glick, S.D., Rossman, S., Steindorf, S., Maisonneuve, I.M. and Carlson, J.N., Effects and aftereffects of ibogaine on morphine self-administration in rats, *Eur. J. Pharmacol.*, 195 (1991) 341-345.
- 13 Greenglass, P. and Bremner, R., Binding characteristics of [^3H]prazosin to rat brain alpha-adrenergic receptors, *Eur. J. Pharmacol.*, 55 (1979) 323-326.
- 14 Hess, E.J., Battaglia, G., Norman, A.B., Iorio, L.C. and Creese, I., Guanine nucleotide regulation of agonist interactions at [^3H]SCH23390 labeled D₁ dopamine receptors in rat striatum, *Eur. J. Pharmacol.*, 121 (1986) 31-38.
- 15 King, S.M. and Tunncliffe, G., Na⁺ and Cl⁻ dependent [^3H]GABA binding to catfish brain particles, *Biochem. Int.*, 20 (1990) 821-831.
- 16 Kosten, T.R. and Kleber, H.D., Buprenorphine detoxification from opioid dependence: a pilot study, *Life Sci.*, 42 (1988) 635-641.
- 17 Lahti, R.A., Mickelson, M.M., McCall, J.M. and Von Voigtlander, P.F., [^3H]U-69593 a highly selective ligand for the opioid κ receptor, *Eur. J. Pharmacol.*, 109 (1985) 281-284.
- 18 Lefkowitz, R.J., Mukherjee, C., Coverstone, M. and Caron, M.G., Stereospecific [^3H]-($-$)-alprenolol binding sites, beta-adrenergic receptors and adenylate cyclase, *Biochem. Biophys. Res. Commun.*, 60 (1974) 703-709.
- 19 Lyon, R.A., Teitler, M., Frost, J.J., Whitehouse, P.J., Wong, D.F., Wagner, Jr., H.N., Dannals, R.F., Links, J.M. and Kuhar, M.J., [^3H]3-N-Methylspiperone labels D₂ dopamine receptors in basal ganglia and 5₂ serotonin receptors in cerebral cortex, *J. Neurosci.*, 6 (1986) 2941-2949.
- 20 Maisonneuve, I.M., Keller, Jr., R.W. and Glick, S.D., Interactions between ibogaine, a potential anti-addictive agent, and morphine: an in vivo microdialysis study, *Eur. J. Pharmacol.*, 199 (1991) 35-42.

- 21 Mello, N.K., Mendelson, J.H., Bree, M.P. and Lukas, S.E., Buprenorphine suppresses cocaine self-administration by Rhesus monkeys, *Science*, 245 (1989) 859-862.
- 22 Miller, K., Weisberg, E., Fletcher, P.W. and Teitler, M., Membrane-bound and solubilized brain 5HT₂ receptors: improved radioligand binding assays using bovine area postrema or rat cortex and the radioligands [³H]GR65630, [³H]BRL43694 and [³H]LY278584, *Mol. Pharmacol.*, in press.
- 23 Muller, W.E., Fehske, K.J., Borbe, H.O., Wollert, U., Nanz, C. and Rommelspacher, H., On the neuropharmacology of Harmane and other β -carbolines, *Pharmacol. Biochem. Behav.*, 14 (1981) 693-699.
- 24 Narajo, C., Psychotherapeutic possibilities of new fantasy-enhancing drugs, *Clin. Toxicol.*, 2 (1969) 209-224.
- 25 Naranajo, C., Psychotropic properties of harmala alkaloids. In D. Efron, B. Holmstedt and N. Kline (Eds.), *Ethnopharmacologic Search for Psychoactive Drugs*, Raven, New York, 1979, pp. 385-396.
- 26 Obata, T. and Yamamura, H.I., The effect of benzodiazepine and β -carbolines on GABA-stimulated chloride influx by membrane vesicles from the rat cerebral cortex, *Biochem. Biophys. Res. Commun.*, 141 (1986) 1-6.
- 27 Payne, G.T. and Soderlund, D.M., Allosteric enhancement by DDT of the binding of [³H]batrachotoxinin A-20- α -benzoate to sodium channel, *Pestic. Biochem. Physiol.*, 33 (1989) 276-282.
- 28 Roberts, E., Wong, E., Svenneby, G. and Degener, P., Sodium-dependent binding of GABA to mouse brain particles, *Brain Research*, 152 (1978) 614-619.
- 29 Rommelspacher, H., Nanz, C., Borbe, H.O., Fehske, K.J., Muller, W.E. and Wollert, U., Benzodiazepine antagonism by harmane and other β -carbolines in vivo and in vitro, *Eur. J. Pharmacol.*, 70 (1981) 409-416.
- 30 Rout, B.R. and Snyder, S.H., [³H]Para-amino-clonidine: a novel ligand which binds with high affinity to α -adrenergic receptors, *Life Sci.*, 25 (1979) 769-774.
- 31 Schulz, D.W., Wyrick, S.D. and Mailman, R.B., [³H]SCH23390 has the characteristics of a dopamine receptor ligand in the rat central nervous system, *Eur. J. Pharmacol.*, 106 (1985) 211-212.
- 32 Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C., Measurement of protein using bicinchoninic acid, *Anal. Biochem.*, 150 (1985) 76-85.
- 33 Titeler, M., Lyon, R.A., Davis, K.H. and Glennon, R.A., Selectivity of serotonergic drugs for multiple brain serotonin receptors: the role of [³H]-DOB, a 5HT₂ agonist radioligand, *Biochem. Pharmacol.*, 36 (1987) 3265-3271.
- 34 Teitler, M., Lyon, R.A. and Glennon, R., Radiolabeled binding evidence implicates the brain 5HT₂ receptor as a site-of-action for LSD and phenylisopropylamine hallucinogens, *Psychopharmacology*, 94 (1988) 213-216.
- 35 Teitler, M., Lyon, R.A., Kuhar, M.J., Frost, J.F., Dannals, R.F., Leonhardt, S., Bullock, A., Rydelek, L.T., Price, D.L. and Stuble, R.G., μ -Opiate receptors are selectively labelled by [³H]carfentanil in human and rat brain, *Eur. J. Pharmacol.*, 167 (1989) 221-228.
- 36 Trouvin, J.H., Jacquemin, P., Rouch, C., Lesne, M. and Jacquot, C., Benzodiazepine receptors are involved in tabernanthine-induced tremor: in vitro and in vivo evidence, *Eur. J. Pharmacol.*, 140 (1987) 303-309.
- 37 Vanderheyden, P., Ebinger, G. and Vauquelin, G., Different agonist binding properties of M1 and M2 muscarinic receptors in calf brain cortex membranes, *Biochem. Pharmacol.*, 36 (1987) 4119-4124.
- 38 Vargus, F. and Erlij, D., The effects of harmaline on GABA fluxes in pinched-off nerve endings, *Brain Research*, 113 (1976) 611-615.
- 39 Villar, H.O., Uyeno, E.T., Toll, L., Polgar, W., Davies, M.F. and Loew, G.H., Molecular determinations of benzodiazepine receptor affinities and anticonvulsant activities, *Mol. Pharmacol.*, 36 (1989) 589-600.
- 40 Zetler, Singbartl, G. and Schlosser, L., Cerebral pharmacokinetics of tremor-producing harmala and iboga alkaloids, *Pharmacology*, 7 (1972) 237-248.